Peer Review
Experimental Toxicology Division
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MODELING

Development of dose metrics for species extrapolation for Persistent Bioaccumulative Toxicants (PBTs)

Janet Diliberto, Michael DeVito, Christopher Hurst[1], David Ross, Vicki Richardson, Frances McQuaid[2], Woody Setzer, and Linda Birnbaum

[1]Curriculum in Toxicology, University of North Carolina, Chapel Hill, NC - [2] Senior Environmental Employment Program

INTRODUCTION

- Default approaches to extrapolating exposures across species use allometric scaling. This approach works very well for pharmacokinetic parameters, such as half-life, for water soluble chemicals.
- For persistent bioaccumulative toxicants (PBTs), the evidence suggests that pharmacokinetic parameters, such as half-life, do not scale well using the default allometric techniques.
- For example, the half-life of TCDD is 15-25 days and 5-11 years, in rats and humans, respectively.
- Thus, default methods for scaling animal exposures to humans are not appropriate and the development of alternative methods are required.

OBJECTIVE

- To develop dose metrics for PBTs for use in cross species extrapolations.

MOUSE MODEL: DOSE METRIC FOR REVERSIBLE BIOLOGICAL RESPONSES

HYPOTHESIS

- Tissue concentration of TCDD is a predictive response for reversible biochemical changes.

APPROACH

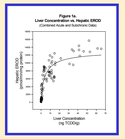
- Examined effects of TCDD on enzyme induction in mice following either single or repeated exposures.
- Acute Study (1): Female B6C3F1 mice: single oral dose (0, 0.1, 1, or 10µg [3H]-TCDD/kg), killed 7, 14, 21, or 35 days post treatment.
- Subchronic Study (2): Mice orally dosed 5 da/wk for 4, 8, 13, or 17 wks (0, 1.5, or 150 ng [3H]-TCDD/kg/da. 13-wk time point: additional doses (0.15-450). Some mice dosed for 13 wks to either 1.5 or 150 followed by 4 wks with no dosing.
- In both studies, TCDD concentrations in blood and liver and hepatic EROD (marker for CYP1A1) enzymatic activity were determined.
- Hill Model (Sigma Stat for Windows; Jandel Scientific Software) was fit to the enzymatic data using either administered dose, daily dose, or tissue concentration as dose metric.
- Hill Equation: $E = Eo + (E\max * Xn) / (bn + Xn)$.
  Where: E=enzyme activity at dose X; Eo=enzyme activity at X=0; X=dose (either administered dose, tissue or body burden; b=ED50; n=Hill shape parameter.

RESULTS

- When tissue concentration or body burdens (BB) were used as dose metric, the Hill model fits resulted in R>0.88 and p<0.001 for all sets analyzed (see Table).
- Daily dose or administered dose did not provide consistent dose response relationships when the acute and subchronic data were combined.
- Using either TCDD tissue concentration or BB as dose metric: similar estimates of ED50 when acute or subchronic EROD data were analyzed separately or combined.
- Similar results were observed for lung and skin EROD activity and hepatic ACOH activity (a marker for CYP1A2).

CONCLUSIONS

- Despite different exposure regiments, tissue CYP1A activities reflected tissue TCDD concentrations and BBs.
- These studies suggest tissue concentrations and BBs are useful dose metrics for describing DR relationships for reversible biochemical responses to dioxins.
- When BB is used as measure of dose, humans are as sensitive as experimental animals for such endpoints as cancer, chloracne, and induction of CYP1A1 (3).
- Estimation of the appropriate dose metric is important in facilitating human risk characterization of adverse health effects to dioxins and dioxin-related PBTs.



TCDD Dosemetric vs. Hepatic Enzymatic Activity (EROD)

	Hill Shape Parameter	ED50
Liver Concentration		
Acute Study	0.771 ± 0.074	3.814 ± 0.477
Subchronic Study: Dose Response (DR)	1.991 ± 0.224	2.637 ± 0.150
Subchronic Study: Time Course (TC)	1.001 ± 0.198	6.482 ± 0.855
Subchronic Study: DR and TC	0.973 ± 0.110	4.848 ± 0.543
Combined Acute and Subchronic Studies	0.863 ± 0.064	4.325 ± 0.367
Blood Concentration		
Acute Study	1.017 ± 0.101	0.0108 ± 0.00110
Subchronic Study: Dose Response (DR)	2.733 ± 0.341	0.0058 ± 0.0003
Subchronic Study: Time Course (TC)	1.470 ± 0.285	0.0131 ± 0.0013
Subchronic Study: DR and TC	1.306 ± 0.106	0.0105 ± 0.00105
Combined Acute and Subchronic Studies	1.163 ± 0.094	0.0106 ± 0.0008
Body Burden		
Acute Study	0.970 ± 0.088	529.6 ± 52.5
Subchronic Study: Dose Response (DR)	5.035 ± 0.937	263.0 ± 8.4
Subchronic Study: Time Course (TC)	1.266 ± 0.250	689.4 ± 76.4
Subchronic Study: DR and TC	1.172 ± 0.147	540.1 ± 58.0
Combined Acute and Subchronic Studies	1.070 ± 0.086	532.8 ± 40.3

Note: Data expressed as mean ± SE.
ED50: Liver and Blood Concentrations exporessed as ng TCDD/g tissue.
ED50: Body Burden expressed as ng TCDD/kg body wt.

REFERENCES

(1) Diliberto *et al.*, 1995. *Toxicol. Appl. Pharmacol.* 130, 197-208.
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RAT MODEL: DOSE METRIC FOR ADVERSE REPRODUCTIVE AND DEVELOPMENTAL EFFECTS

HYPOTHESIS

- Fetal tissue concentration of TCDD is sufficient to predict the severity of development abnormalities.

APPROACH

- Model fitting involved incidences of various abnormalities for a range of maternal exposures on either GD 8 or GD 15.
- Maternal and fetal TCDD concentrations: Determined for same administered dosages in separate experiments (1).
  - Time-pregnant Long Evans rats: Single oral dose on GD8 of 1.15 µg[3H]TCDD/kg or on GD15 of 0.05, 0.20, 0.80, or 1.0 µg[3H]TCDD/kg; TCDD concentrations in maternal and fetal tissues measured on GD16 and GD21.
  - Response data following GD8 or GD15 exposure to TCDD (2,3,4,5). Tissue concentrations after GD8 exposure (6).
- Dose-response model: For each developmental abnormality, males: ejaculated sperm counts & delayed puberty; females: urethra-phallus distance & incidence of vagina thread.

RESULTS

- Fetal TCDD tissue concentrations on GD16 provides good prediction of change in ejaculated sperm counts and puberty delay in males and urethra-phallus distance in females following exposure on GD8 or GD15.
- Fetal TCDD tissue concentrations on GD16 underpredict the incidence of vaginal threads following GD8 exposures.
- Administered dose poorly predicts developmental responses.

CONCLUSIONS

- Fetal tissue concentrations at a critical period of sensitivity is an appropriate dose metric for predicting TCDD developmental effects.
- Concentrations of TCDD in developing fetus are highly correlated with concentrations found in maternal blood.
- Thus, measurement of maternal blood levels at a critical time provides a means to estimate fetal exposure to dioxin and potential effects associated with this exposure.
- A better understanding of the relationship between tissue concentration of TCDD and development of adverse outcomes may facilitate ability to predict whether human populations are at risk for effects associated with low-level exposure to TCDD.

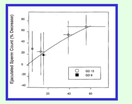

Fig. 1. Percent decrease in ejaculated sperm count vs. estimated mean fetal TCDD conc. on GD16. Tissue concentrations provide good prediction of effect.

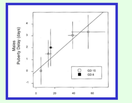

Fig. 2. Puberty delay in males vs. estimated mean fetal conc. on GD16. Tissue concentrations provide good prediction of effect.

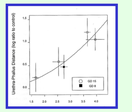

Fig. 3. Female urethra-phallus distance plotted versus log fetal tissue conc. on GD16. Tissue concentrations provide good prediction of effect.

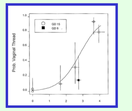

Fig. 4. Incidence of vaginal thread vs. log tissue conc. of GD16. Tissue concentrations are poor predictors for this response

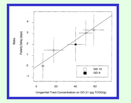

Fig. 5. Mean puberty delay in males plotted vs. mean urogenital tract TCDD conc. on GD21. Tissue concentration provides good prediction of effect.

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CONCLUSIONS

- Body burdens of TCDD are proportional to tissue concentrations.
- Tissue concentrations are good predictors of response across exposure regiments for two different responses in two different species. This provides support for the use of body burdens as a dose metric for cross-species extrapolations.

FUTURE DIRECTIONS

- It is uncertain whether body burden is the most appropriate dose metric for cancer.
- Future studies are needed to validate body burden as the most appropriate dose metric for cancer.
- In addition, extension of this work to other PBTs would aid in testing the use of body burden as a dose metric for all PBTs.

IMPACT

- Bases on this work, the USEPA has proposed using steady-state body burdens as the dose metric for cross species extrapolations.
- Other public health agencies, including WHO, Ministry of the Environment of Japan, and JEFCA have applied body burden as their cross species dose metric for dioxins.
- Use of body burdens as a dose metric allows for extrapolations without the need for an uncertainty factor for pharmacokinetics.

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